Li et al. *Zool. Res.* 2024, 45(3): 464−467
https://doi.org/10.24272/j.issn.2095-8137.2023.232

ZR | Zoological Research

Letter to the editor Open Access

Haplotype-resolved and chromosome-scale genomes provide insights into co-adaptation between the Amur tiger and Amur leopard

DEAR EDITOR,

Big cats, such as Amur tigers (*Panthera tigris altaica*) and Amur leopards (*P. pardus orientalis*), are apex predator and have evolved specialized traits for hunting and carnivory (Moya et al., 2022), thus playing a crucial role in maintaining biodiversity and ecosystem integrity by regulating prey-predator dynamics. However, human-induced pressures, habitat fragmentation, and environmental alterations have restricted these species in small and isolated populations. Currently, all extant big cats are categorized as endangered or threatened according to their conservation status. Amur tigers and Amur leopards share overlapping geographic ranges, habitats, and certain prey species in the forests of Northeast Asia (Jiang et al., 2015). To reduce interspecies conflict, these carnivores exhibit differentiated dietary and temporal niches. Amur tigers predominantly prey on large ungulates, while Amur leopards hunt small to medium-sized animals (Sugimoto et al., 2016). Additionally, they occupy different temporal niches, with tigers being active at night and leopards more active during the day. Despite spatial and temporal niche partitioning, interspecific competition between these two species is inevitable. Tigers, benefiting from their greater size, have a competitive advantage over leopards, which can manifest in occasional leopard predation by tigers and declines in leopard populations with increasing tiger density (Jiang et al., 2015). Tigers also displace leopards from marginal habitats in nature reserves where they coexist.

The coexistence and competition between these big cats involve not only ecological factors but also genetic factors underlying physiological traits. However, the genetic basis for co-adaptation and competition has not been well researched. In this study, we assembled a haplotype-resolved and chromosome-scale genome for a wild Amur leopard without parental information and conducted comparative genomic analysis with the Amur tiger genome to elucidate the genetic basis for coexistence and competition. This research has implications for the future conservation of these two endangered species.

Comparative genomics aims to understand the similarities and differences between genomes of different organisms. Genome assembly provides the necessary scaffolding for downstream comparative genomic analyses, facilitating the identification of genetic variations and the exploration of functional elements in the genome. Based on blood samples collected from a rescued Amur leopard in the wild, we successfully assembled a high-quality genome for this species (hereafter PpoHapG), with a genome size of 2.42 Gb and a scaffold N50 value of 146.59 Mb (Figure 1A, B; Supplementary Tables S1, S2). More than 2.40 Gb of scaffolds (~99.34%) were anchored to 19 chromosome-scale pseudomolecules with a complete X chromosome identified (Supplementary Figure S1 and Table S3). The hybrid genome was then phased using a Hifiasm assembler into two groups of haplotigs, with 19 chromosomes generated in each set of haplotigs (hereafter referred to as PpoHapGH1 and PpoHapGH2, respectively) (Supplementary Figure S2). Genome completeness, measured by Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis, showed high scores for all three genomes, while base-level quality evaluation indicated that all three genomes had high QV scores (Supplementary Tables S4, S5). Subsequently, we conducted a comprehensive analysis to identify repetitive sequences and protein-coding genes (Supplementary Figure S3 and Tables S6–S10). Additionally, collinearity analysis exhibited consistency in the leopard genome, both within haplotypes and among closely related species (Supplementary Figures S4, S5). Furthermore, the published Amur tiger genome (hereafter PtaHapG) was used for subsequent comparative analyses (Lan et al., 2023).

The genomes were complete, with few haplotype-specific sequences. In total, 2 586 structural variants were identified, including deletions, duplications, translocations, and inversions, supported by genome mapping (Figure 1A; Supplementary Table S11). These structural variants affected genes related to thyroid and parathyroid function, heart function, insulin secretion, growth hormone synthesis, and olfactory receptors (Supplementary Figures S6, S7 and Table S12). Only a few pseudogenic genes were specific to one haploid genome, related to reproduction and energy metabolism (Supplementary Table S13).

To investigate the genomic basis of the biological characteristics of the tiger and leopard as apex predators, we

Received: 11 October 2023; Accepted: 25 December 2023; Online: 26 December 2023

Foundation items: This study was supported by the Fundamental Research Funds for the Central Universities of China (2572022DQ03), National Natural Science Foundation of China (32170517), and Guangdong Provincial Key Laboratory of Genome Read and Write (2017B030301011). This work was also supported by China National GeneBank (CNGB)

first constructed a phylogenetic tree and performed gene expansion analysis. In total, 171 and 280 gene families were expanded in the PtaHapG and PpoHapG genomes, respectively (Supplementary Figure S8). Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses revealed similar enriched terms and pathways shared by the two species, with energy metabolism-related GO terms predominant in both PtaHapG and PpoHapG (Supplementary Figures S9–S12). Interestingly, long-term potentiation (LTP) (map04720) was significantly enriched in the expanded gene families in the PtaHapG genome based on KEGG pathway analysis (Figure 1C). LTP is a long-lasting increase in the strength of connections between neurons in the hippocampus and is thought to be a cellular molecular mechanism underlying learning and memory (Dong et al., 2015). In this pathway, the calmodulin (*CaM*) gene family was expanded in both the PtaHapG and PpoHapG genomes relative to other Felidae species (Figure 1D). CaM is a ubiquitous Ca^{2+}-binding protein, and the Ca^{2+}/CaM complex activates Ca^{2+}/CaM-dependent kinase II (CaMKII), which is critical for the synaptic plasticity underlying learning and memory (Chang et al., 2019). Thus, expansion of the *CaM* gene family may enhance *CaM* gene expression and potentially impact LTP pathway functionality in the Amur tiger.

The analysis of positive selection in comparative genomics is commonly employed to unravel adaptive evolution and comprehend the genetic basis of species-specific traits. In this study, we identified 119 positively selected genes (PSGs) and 401 rapidly evolving genes (REGs) in PtaHapG and 139 PSGs and 370 REGs in PpoHapG. In both the PtaHapG and PpoHapG genomes, many of the identified genes were closely related to the physical requirements of big cats as apex predators (Supplementary Tables S14, S15), including energy metabolism, vision, muscle, teeth and bone, immunity, lipid metabolism, cardiovascular, and other shared PSGs related with hearing, smell, claws, and adrenal glands (Figure 1E; Supplementary Figure S13). Similarly, more species-specific amino acid changes were found in the tiger genome compared to the leopard genome (Figure 1F–1H). Strikingly, the REG *MEF2C* was identified in PtaHapG, but not in PpoHapG (Figure 1E). This gene negatively regulates excitatory synapse number and function to further modulate basal and evoked synaptic transmission, facilitating hippocampal-dependent learning and memory. In mice, overexpression of *MEF2C* in the adult prefrontal cortex improved working memory and cognition, similar to the effects observed with the *KMT2A* gene (Supplementary Table S16). Similarly, *ADCY9* was found to be under strong positive selection in the PtaHapG genome but not in the PpoHapG genome. The expression of this gene was reduced in the hippocampus of aged mice but increased after spatial learning, suggesting its potential involvement in regulating learning, memory, and cognition (Supplementary Table S16). Thus, the presence of the *MEF2C* and *ADCY9* genes suggests that the tiger may have superior cognitive ability to the leopard. Of note, the tiger boasts a larger brain size compared to leopards and other large felines, harboring pyramidal neurons in the prefrontal, motor, and visual cortices. These pyramidal neurons exhibit more complex and disproportionately larger dendritic structures relative to their body/brain size. Such features are pivotal for higher cognitive functions, including planning, problem-solving, reasoning, episodic memory retrieval, musculoskeletal functions, and visual information processing (Supplementary Table S16). These observations, together with our research findings, provide a genetic basis for the biological differences between Amur tigers and Amur leopards.

Given that the Amur tiger and Amur leopard share a recent common ancestor and largely overlap in ecological niches as apex predators within coexisting habitats, we expected genomic signatures of parallel evolution to emerge between them, distinct from other species. Notably, we identified 17 parallel evolutionary genes between PtaHapG and PpoHapG, many of which were closely related to survival in the wild, including wound healing (*PEAR1* and *MEGF10*), bone development and healing (*FARP2*), hearing (*PKHD1L1*), and muscle development and energy metabolism (*SLC25A25* and *MYO18B*) (Supplementary Table S17 and Figure S14). These findings suggest that the Amur tiger and Amur leopard have undergone parallel evolution in the aforementioned aspects, aligning with the physiological demands of apex predators. To further explore adaptively parallel genes (APG), we screened the 17 genes that were simultaneously under positive selection, among which one APG (*SLC25A25*) and five APGs (*FREM2*, *L1CAM*, *MEGF10*, *PEAR1*, and *SLC5A9*) were identified in the PpoHapG and PtaHapG genomes, respectively (Figure 1E). The *KMT2A* gene, required for the formation of working memory and memory consolidation (Jakovcevski et al., 2015; Kerimoglu et al., 2017), emerged as a parallel evolutionary marker in both genomes. Working memory is related to intelligence, information processing, executive function, comprehension, reasoning, problem-solving, planning, and learning in humans and animals (Cowan, 2014). As such, these observations imply that the Amur tiger and Amur leopard may exhibit superior cognitive abilities compared to other Felidae species.

This study provides a comprehensive understanding of the competition between Amur tigers and Amur leopards through comparative genomics analysis. These two big cat species, occupying the same niche as top predators, evolved in parallel with shared genomic characteristics related to hearing, smell, claw and muscle formation, immunity, reproduction, and detoxification. These shared functions underpin their predatory efficiency and survival abilities. Furthermore, we identified specific genes and amino acid changes in the two species associated with bone health, cardiovascular function, reproductive capability, and immune response. Overall, our findings indicate that both species have evolved similar physiological traits to adapt to their current habitats, allowing for their coexistence and interspecies competition.

However, our study also uncovered genomic distinctions that provide tigers with a competitive advantage over leopards. Notably, the Amur tiger possessed genes related to olfactory receptors, reproduction, and energy metabolism, which were absent in the leopard, suggesting weaker functionality in these aspects for the latter. Additionally, the tiger benefited from expansions in gene families associated with food digestion, mineral absorption, and vision. We also identified tiger-specific amino acid changes in certain genes, which potentially enhanced their adaptability as an apex predator. The tiger also exhibited a higher number of immune- and cardiovascular-related genes compared to the leopard, suggesting superior adaptability in these domains. Conversely, the leopard possessed a higher number of energy metabolism-related genes, indicative of adaptations to a higher metabolic demand due to its smaller body size (White & Seymour, 2003). Furthermore, comparative analysis of the

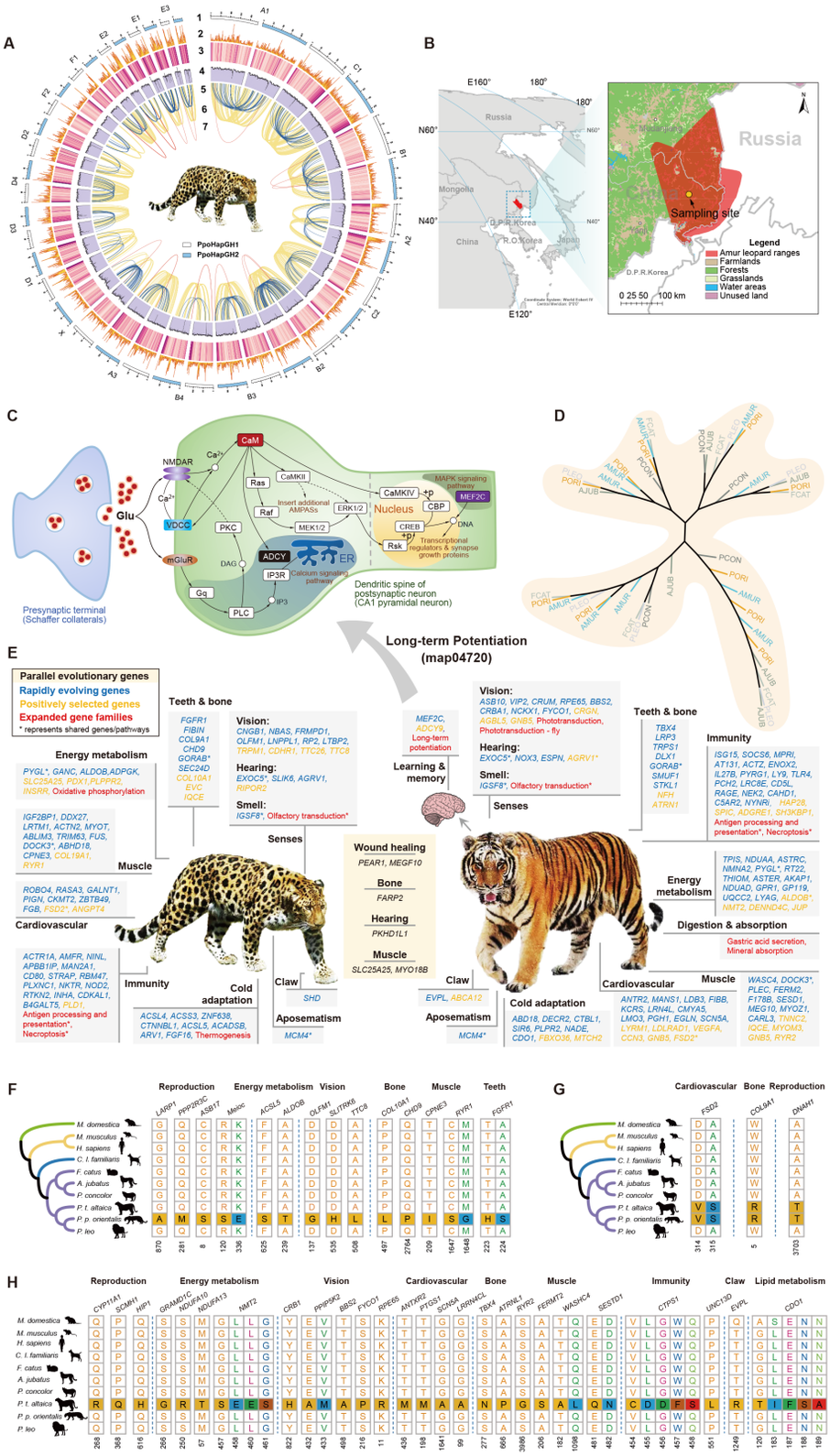

Figure 1 Genomic landscape and basis for the co-adaptation of Amur tiger and Amur leopard

A: Genomic characteristics of Amur leopard. 1. Schematic of chromosomes (white: PpoHapGH1; blue: PpoHapGH2); 2. Gene density; 3. Sequencing depth; 4. GC content; 5. SV: duplication; 6. SV: inversion; 7. SV: translocation. B: Distribution area and sampling sites of Amur leopard. C: Long-term potentiation pathway. *CaM* gene family was significantly expanded in both Amur tiger and Amur leopard genomes. Rapidly evolving gene *MEF2C* and positively selected gene *ADCY* are in purple and black, respectively. D: Maximum-likelihood phylogenetic tree of *CaM* gene family. Amur tiger genes are highlighted in light blue, Amur leopard and other Felidae species genes are in orange and gray, respectively. E: Genes and pathways with either shared or unique evolutionary signals in Amur tiger and Amur leopard and their putative adaptive functions. F: Amur leopard-specific amino acid changes in REGs compared to other species. G: Amino acid changes in REGs shared by Amur tiger and Amur leopard. H: Amur tiger-specific amino acid changes in REGs compared to other species.

leopard genome revealed a higher enrichment of disease-related pathways compared to the tiger, hinting at a potentially greater susceptibility to certain diseases in leopards.

Overall, our study provides a comprehensive and well-annotated genomic analysis of the Amur leopard and Amur tiger, revealing a greater presence of genomic signatures associated with top-predator traits in the tiger compared to the leopard, thus shedding light on their one-sided competition from a genetic perspective. To ensure the co-conservation of these top predators, strategies that facilitate effective niche partitioning should be implemented. Furthermore, understanding ecological thresholds such as climate change, habitat availability, vegetation, prey base, competitors, and anthropogenic disturbance could help inform management approaches that promote habitat resources favoring niche partitioning between tigers and leopards.

DATA AVAILABILITY

The data supporting the findings in this study have been deposited in the CNGB Sequence Archive (CNSA) of the China National GeneBank Database (CNGBdb) under accession number CNP0003803 and Science Data Bank (DOI: 10.57760/sciencedb.j00139.00119).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

T.M.L., H.M.L., Y.C.X., and G.S.J. initiated and designed the project. D.L., Y.Z., W.Y.K., Y.M., B.Y.L., and L.Z. collected the samples. L.Y.C. and S.C.Y. performed RNA/DNA isolation. H.R.L. performed DNA library construction and sequencing. T.M.L. coordinated the data analysis. H.M.L., M.H.S., and B.Y.L. carried out the data analysis. T.M.L., H.M.L., B.Y.L., and M.H.S. wrote the manuscript. Y.C.X., S.K.S., S.L.L., N.D., L.D., H.L., and G.S.J. revised the manuscript. Y.C.X. and G.S.J. supervised the project. All authors read and approved the final version of the manuscript.

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